**Figure S1.** Schematic diagram for designing sgRNAs

**Figure S2.** Cas9 cleavage assay for checking sgRNA efficiency in vitro. Digesting templates were genomic DNA amplified using the specific primers for each prolamin gene subgroups (Pro13a-I and Pro13b-I/II) and the testing sgRNAs were transcribed *in vitro* (using the Guide-it Complete sgRNA Screening System). Symbol (+) and (-) indicate the presence or absence of sgRNA-Cas9 complex, respectively. S/M: size marker. Fragments of cleaved-Pro13a-I are 210bp and 190bp. Fragments of cleaved Pro13b-I/II are 250bp and 180bp.

**Figure S3**. Construction of a CRISPR-Cas9 binary vector for editing rice 13 kDa prolamin genes.

**Figure S4.** Genotype of *13 kDa prolamin*-knockout lines in T0 generation. (A) Sequence analysis of target genes in T0 plants showing various indel mutations. Sequences of sgRNAs were marked with underlines, PAM sequence was marked in boxes; dash lines and bold letters represent deletions and insertions of nucleotides, respectively; and ‘\*’ represents nucleotides identical to those in the original WT sequences. (B) Transgenicity assay. The gel image represents PCR amplification of 1839-bp *SpCas9* gene and a 615-bp *HygR* gene in the putative T0 transgenic plants. S/M: size marker.

**Figure S5.** Transgenicity assay of T1 plants. The gel image represents PCR amplification of 1839-bp *SpCas9* gene and a 615-bp *HygR* gene in the putative T1 transgenic plants. S/M: size marker

**Figure S6.** Regression plot showing strong positive linear relationship between RNA seq data and qRT-PCR data with an R-square > 0.8. The qRT-PCR data is normalized using 2-ΔΔCT method and the RNA seq transcript is presented as log2[foldchange] data. The plot was visualized using SRplot “Pearson spearman scatte” (https://www.bioinformatics.com.cn/plot\_basic\_pcc\_scatter\_plot\_049\_en).